U.S. NONPROVISIONAL PATENT APPLICATION

METHOD OF PRESERVING FRESH PERISHABLES

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METHOD OF PRESERVING FRESH PERISHABLES

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CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application is based on and claims priority from U.S. Provisional Application No. 60/442,980, filed January 28, 2003, and U.S. Provisional Application No. 60/503,062, filed September 15, 2003, both of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[002] The shelf life of most perishables, including respiring produce, non-respiring prepared or cooked perishables and raw or cooked muscle foods can be extended by the application of various gas/vapor mixtures. These mixtures are commonly referred to as Modified or Controlled Atmospheres (MA/CA). Other acronyms include MAP, which refers to packaging applications, as contrasted with storage or transportation applications.

[003] Where microbial spoilage is the primary cause of reduced shelf life, microbial-static and microbial-cidal gases and vapors (such as CO_2) are effective agents for extending the microbial shelf life of such perishables. However, complications arise when the most effective levels of these agents also cause damage to the color, flavor, odor and texture of the perishable of interest or to one of the perishables in a mixture of interest. Consequently, these agents are frequently not used or are used at sub-optimal levels resulting in shorter shelf life. Shorter shelf life frequently results in higher production and distribution costs along with higher spoilage losses and increased potential for product failure at the end user (i.e., a customer unhappy with the product).

[004] Food safety issues have also been responsible for the limited application of effective (low oxygen) MA/CA mixtures for those foods that are susceptible to the growth of *Clostridium botulinum* and the resulting food-borne illness termed botulism.

SUMMARY OF THE INVENTION

[005] The present invention relates to a process for packaging perishable food items, particularly fresh cut fruit, comprising the steps of: (a) placing fresh cut food pieces in a package at least a portion of which is gas permeable; and (b) adding an antimicrobial gas (preferably carbon dioxide) into said package at a level of from about 20% to about 100% (most preferably from about 75% to about 100%) of the atmosphere contained within the package;

wherein said package has a permeability such that the atmosphere in the package equilibrates with the atmospheric gas composition in about 1 to about 7, preferably about 2 to about 4, days from the time the antimicrobial gas is added to the package, at from about 28°F to about 212°F, preferably from about 32°F to about 50°F.

[006] In addition, the present invention relates to a package for holding fresh cut fruit during storage and/or transportation, at least a portion of which package is gas permeable, and which is structurally adapted to hold an initial level of antimicrobial gas (preferably carbon dioxide) of from about 30% to about 100% of the atmosphere contained within the package; and wherein said package permits the atmosphere in said package to equilibrate to no more than about 20% antimicrobial gas in from about 1 to about 5 days at about 28°F to about 212°F, preferably about 32° to about 50°F.

DETAILED DESCRIPTION OF THE INVENTION

[007] The present invention relates to a novel method of preserving fresh perishables, such as fresh cut fruit, so as to retard spoilage and extend freshness. This invention extends the freshness of foods, especially fresh cut produce, by surrounding the food for a controlled (limited) time period with CO_2 or other antimicrobial gases or vapors at levels well in excess of the maximum levels widely accepted by those skilled in the art to be permanently damaging to the food's characteristic flavor, color, odor or texture. The method of the present invention applies to both whole or cut produce, either packaged alone or together with any other (non-produce) food product.

[008] The method is particularly useful with low acid fruit, such as melons (for example, watermelon, cantaloupe, honeydew, etc.), although it may also be used on virtually any other type and mixture of fruit (whole or cut), cooked, raw or fresh perishable as well. The method not only inhibits the growth of spoilage flora on the fruit, but it also inhibits the loss of flavor and texture which generally takes place with fresh cut fruit over time. The net result of this method is that fruit can have a fresh cut appearance, smell and taste for as long as 10-14 days (at 45°F), or possibly even longer, after it is cut. This level of high quality shelf life for fresh cut fruit has been unattainable to date (without the use of preservatives) by the fresh cut fruit industry using known and available technology. This invention is typically used in conjunction with high quality raw materials and a sufficiently sanitary process that insures the initial microbial load on the perishable or fresh cut fruit is minimized.

[009] The method is accomplished by placing the perishable(s) in a package or container or enclosure made up, in whole or in part, of microperforated, microporous or differentially gas permeable materials (for example, membranes, tray lidding, bags, master bags, refrigerated containers, controlled atmosphere (CA) storage rooms or any size enclosure that is capable of achieving and maintaining specifically defined modified atmospheric conditions (such as storage rooms, ship holds, rail cars, or ship or truck containers)) for times necessary to accomplish the benefits of the invention taught herein. As used herein, the term "package" is intended to have that broad definition. Sufficient carbon dioxide or any other antimicrobial gas or volatile material (for example, chlorine oxide, ozone, ethanol, nitrous oxide, carbon monoxide, peroxide) is introduced into the package so as to temporarily or permanently inhibit the growth and/or kill undesirable microorganisms associated with the perishable (fruit) present in the package. Carbon dioxide is preferred. Initially high levels of, for example, CO_2 provide significantly greater inhibition of growth and killing of spoilage microorganisms than are attainable with the same perishables stored or packaged in conventional MA or CA environments, or air. This element facilitates the present invention's ability to maintain freshness and inhibit spoilage of perishables for extended periods of time. Some gases (particularly carbon dioxide) provide additional benefits with fresh produce and fresh cut produce, such as

inhibiting ethylene damage to the fruit and slowing down respiration rate thereby extending fresh odor, flavor, color and texture.

[010] Two important findings, among others, form the underpinnings of the present invention. One is the shelf life longevity which occurs when susceptible perishables (e.g., fresh cut fruit) are exposed to high levels of antimicrobial material (e.g., CO_2) for a relatively short duration. The second is that temporary exposure of damage susceptible perishables to higher than recommended levels of antimicrobial volatiles results only in <u>temporary</u> damage to those perishables. The prior art would have suggested that such damage would have been permanent and, therefore, would not have contemplated such exposure.

[011] The high levels of carbon dioxide or other antimicrobial agent may be introduced into the package or container by vacuum back flush, injection, permeation or any other suitable means. An important aspect of this invention is the use of initial levels of carbon dioxide (or other antimicrobial agent) that are above those reported to be injurious to the perishables of interest. For example, CO_2 levels greater than 15% on fresh produce are widely reported to cause off-flavor and injury. In this invention, CO_2 is introduced at from about 20% to about 100% (preferably from about 30%, more preferably from about 40%, more preferably from about 50%, still more preferably from about 60%, up to about 100%) of the atmosphere contained in the package.

[012] Gas permeable packaging or mechanically actuated leakage or evacuation facilitates controlled release (dissipation) of the antimicrobial gas (e.g., CO_2) level in the package such that it equilibrates to a more typical Modified Atmospheric (or air) composition. In that way, the fruit is not kept under a high (potentially damaging) CO_2 atmosphere for an extended period of time, thereby minimizing damage to the perishable caused by the CO_2 while still damaging or inhibiting spoilage organisms and inhibiting the damaging effects of ethylene. As used herein, "equilibrates to a more typical atmospheric composition" means that the final atmospheric composition in the package approaches that of the atmosphere (especially in terms of CO_2 and O_2 levels) when compared to the initial levels (i.e., the CO_2 level decreases), bearing in mind that respiration of the fruit and longer term of microbial flora present may

significantly affect the CO_2 and O_2 levels. For example, to allow for the dissipation of high CO_2 induced off-flavor prior to consumption, it is preferred (for most fresh cut fruit applications) that the atmosphere in the package start with an atmosphere of at least about 30% CO_2 (more preferably at least about 40% CO_2) and equilibrate to an atmosphere which contains no more than about 25% CO_2 within about 2 to about 4 days. In general, equilibration will take from about 1 to about 7 days, preferably 5 days or less, more preferably from about 2 to about 4 days, at from about 28°F to about 212°F, preferably about 32°F to about 50°F. Generally, in this application, "equilibration" of the package atmosphere is to normal atmospheric conditions. However, the packages could be placed in a storage room having a controlled atmosphere (i.e., an atmosphere different from normal atmospheric composition) in which case the packages would "equilibrate" to the content of that controlled atmosphere.

[013] With this desired result in mind, the precise permeability of the packaging or rate of air leakage may be determined by one skilled in the art. The permeability of the package or controlled air leakage will vary depending upon, for example, the particular gases used, the mixture of perishables or identity of the fruit, fruit mix or perishables mix, the size of the package (head space), the amount and surface area of the fruit or perishables, and the net weight and surface area of the packaging material. The precise initial CO_2 levels and dissipation times will also typically depend, for example, on the nature of the perishables and susceptibility to CO_2 injury over time at a given temperature regime, the distribution time to the end user and the desired shelf life of the product.

[014] Where higher initial CO_2 levels are desired, and distribution times are short or the potential for permanent damage to the perishable high, faster dissipation rates may be used; where lower initial CO_2 levels are used, slower dissipation rates may be used. For example, when ultra-high levels of CO_2 are used (e.g., 50% or higher), even short exposure periods (e.g., fast dissipation rates) of 1 or 2 days may be acceptable for conferring extended shelf life. When levels of CO_2 in the 30% or 40% range are used, lower dissipation rates (i.e., longer dissipation times) may be preferred.

[015] Although the present application has been framed primarily in terms of fresh cut fruit, the method of the present invention can be used with perishables and fresh foods of any kind, and mixtures thereof. For example, applications could include fresh meat, fish and poultry or prepared meals containing a precooked entrée (meat, pasta, vegetable) with or without uncooked fresh cut fruit or other fresh produce. The present invention may also, for example, be used with packages of raw beef. In that instance, the high initial levels of CO_2, for example, will keep the meat from spoiling while allowing oxygen rich air to reenter the package, returning the color of the meat to the desirable red by the time of purchase, without requiring the high costs of modified atmosphere packaging currently used in meat packaging.

[016] The preferred execution of the present invention, however, is with fresh cut fruit, such as pineapple, cantaloupe, honeydew, strawberries, grapes and/or watermelon. A preferred embodiment of the present invention, for use with such fruit, packages the fruit at an initial atmosphere which comprises at least about 50% (preferably about 75%) CO_2. That atmosphere equilibrates such that it contains from about 15% to about 20% CO_2 (preferably about 16-17% CO_2) three days after packaging. This rate and level of equilibration has been demonstrated to result in fresh cut fruit without any significant high CO_2 induced off-flavor by the third day. This time period is targeted to match the normal distribution and earliest consumption window for commercially produced fresh cut fruit. It is preferred that the fruit or other perishables be surface sanitized (for example, by surface washing, irradiation, chlorine dip or the administration of heat (e.g., steam, hot water, hot air, infrared) before they are cut up or packaged in order to minimize the amount of surface flora on the fruit. When CO_2 (particularly high levels) is introduced it may be done at a refrigerated initial temperature, room initial temperature or warm initial temperature. Warm initial temperatures may provide some advantages because of the higher rate of microbe metabolism at such temperatures.

[017] Most commercial producers of fresh cut fruit employ "very low barrier" micro-perforated packaging materials or other materials that facilitate a relatively high rate of gas exchange between the inside and outside of the package compared to differentially permeable "low barrier" or impermeable "barrier" type packaging materials. These very low barrier materials facilitate higher rates of entry of outside

oxygen into the package and release of respiration produced CO_2 out of the package. This "very low barrier" packaging is designed to insure that oxygen equilibrates in the package at high enough levels to prevent an anaerobic environment and a possible botulism incident, especially with low acid type fruits (e.g., melons). The high gas permeability of these materials also prevents the buildup of excessive CO_2 levels that could swell the package or damage the flavor, appearance or texture of the product. This inventor's research has shown that, in general, the shelf life of most fresh cut fruit is compromised by using these very low barrier materials compared to materials that facilitate lower equilibrium oxygen levels and higher equilibrium CO_2 levels. Generally speaking, shelf life has been shown to decline by 20-30% in very low barrier materials compared to higher barrier materials. For fresh cut melons, for example, this translates into 6-8 days in very low barrier as compared with 8-10 days (at 45°F) in higher barrier materials. This packaging-related decline in shelf life can be largely attributed to the inability of very low barrier packaging to retain and equilibrate to recommended beneficial levels of CO_2 (5-15%). This has led many experts in the industry to not use or abandon the use of active gas flushing prior to applying a very low barrier seal or lidding film to fresh cut fruit packages. In fact, there are many experts who claim that gas flushing with elevated CO_2 and/or lower oxygen provides no shelf life benefit for fresh cut fruit.

[018] As illustrated in the following examples, this invention can recover the 20-30% loss of shelf life caused by the food safety requirement of using very low barrier packaging materials. Furthermore, this invention can add 40-75% additional days of shelf life at 45°F using very low barrier packaging materials containing initially higher than typical levels of CO_2.

[019] The significant shelf life extensions facilitated by this invention will break the "short shelf life" paradigm that the fresh cut fruit industry has been operating within to date. Longer shelf life will facilitate new, more competitive cost structures and superior products. It is expected that this invention will also facilitate similar advances in other categories of perishables where cost and quality can benefit from the methods taught herein.

[020] Packaging and containers that can be used to practice this invention include, but are not limited to, rigid, thermoformed containers pre-made or thermoformed in-line, made from plastics such as polyvinylchloride (PVC), polystyrene, polyethylene, and polyethylene terephthalate (PET). These materials may be used alone or in composites, blends, laminates or co-extrusions with other materials. These containers hold amounts of products ranging from ounces to pounds, and are usually closed or sealed with a film heat sealed across the top of the container or a snap-on lid with or without a ribbon of plastic to seal around the edges. Other packaging configurations include flexible bags or pouches made of various plastics either in pre-made bag form or in-line. The barrier properties of these materials can be modified in many ways including controlled leakage, microporosity, micro or macro perforations or other intentional or inherent leakage. Depending on the size of the bag, ounces to tons of perishables may be packaged according to this invention. Bags may be sealed by folding, twist-tying or heat sealing. Other means of controlling gas exchange include differential permeability of the package or container whereby the packaging materials do not have any intentional holes or leaks, but exchange gases according to the permeability or gas transmission properties of the materials employed. For larger scale applications of this invention in storage or transportation modes, suitable containers include existing CA storage rooms, ocean or over-the-road transportation containers or palletized configurations where a full pallet of perishables is enclosed within a plastic bag or suitable shroud. An example of a package which may be used in the method of the present invention is the TECTROL™ pallet bag system, commercially available from TransFresh Corporation, Salinas, California.

[021] The following examples are intended to be illustrative, and not limiting, of the present invention.

Example 1

Experimental Setup

[022] Whole cantaloupe and seedless watermelon were surface sanitized using manual washing and scrubbing with antimicrobial soap followed by a 200 ppm chlorine-in-water dip/rinse for 1 minute. These melons were then hand peeled and cut into ¾ to 1 inch size pieces with sanitized knives. Four ounces each of the cut

cantaloupe and watermelon (total 8 ounces) were weighed into plastic PVC cups laminated with a polybutyl peelable seal layer (from MAP Systems, Chicago, Ill.). These cups were 4.75" tall, with a 4.2" diameter opening. After filling, the cups were divided into 3 treatment groups: 1) 25-30% CO_2, balance air gas flush (MAP 3-C); 2) 50-55% CO_2, balance air gas flush (MAP 5-C); and 3) 70-75% CO_2, balance air gas flush (MAP 6-C). The cups were then sealed according to the above treatments with a micro-perforated lidding film supplied by P-Plus, a division of Amcor Inc. The gas flush, sealing packaging machine was a MAP Systems MS-55 (with vacuum). The P-Plush lidding material (52LD80 368 mm) was made of a polyester-to-polyethylene laminate material with an average of 5 micro-perforations per impression/lid. According to P-Plus test, the measured OTR (oxygen transmission rate) of this film is 419 cc of oxygen per package per day. The OTR of the cup material is unknown and believed to be negligible relative to the OTR of the micro-perforated lidding material. All sample cups were then stored at 45-46°F until the evaluations on days 3 and 7.

Results and Conclusions

[023] As summarized in Table 1, the initial CO_2 levels dissipate rapidly due to the high OTR of the micro-perforated lidding film. Regardless, day 3 CO_2 and oxygen levels correlate with the initial CO_2 gas flush levels. It has been observed in this research that, depending on the initial microbial load, the CO_2 and oxygen levels become increasingly influenced over time by the rate of microbial growth and the generation of CO_2 and consumption of O_2 related to that microbial growth. Consequently, by day 7 it can be seen that the CO_2 and O_2 levels are no longer positively correlated with the initial gas flush level, but more closely related to the degree of microbial growth and resulting spoilage.

[024] The microbial counts in Table 2 show the typical response of microbial spoilage floras to the increasing levels of CO_2. By day 7 these differences have diminished as a consequence of the relatively high initial counts. It has been noted in the course of this work that the lower the initial counts, the longer the inhibition of microbial growth and corresponding shelf life with higher initial CO_2 levels. Preferred initial counts are below about 1000 and preferably below about 500.

[0025] Regardless of the higher than optimal initial microbial counts it can be seen in Table 3 that off-flavor was not a significant problem on day 3, and the quality of the fruit on day 7 was best with the highest initial CO_2. Based on previous work, without MAP or with conventional MAP (</=20% CO_2), the cut cantaloupe and watermelon would have been spoiled (acceptability = 1) between days 3 and 5 at 45°F due to the moderately high initial microbial counts.

[0026] Table 1

Treatment (% CO_2)	Initial		Day 3		Day 7	
	CO_2	O_2	CO_2	O_2	CO_2	O_2
25-30	28.6	13.9	5.6	17.3	17.7	7.4
55-60	57.8	8.0	9.0	16.9	17.8	11.5
70-75	73.4	5.0	11.4	16.4	15.4	8.8

[0027] Table 2

Treatment (% CO_2)	Average Initial		Day 3		Day 7	
	*TPC	**LAC	TPC	LAC	TPC	LAC
25-30	30,500	3,916	21,000,000	5,333,333	1,266,666,666	348,333,333
55-60	30,500	3,916	8,766,666	4,130,000	1,353,333,333	340,000,000
70-75	30,500	3,916	5,933,333	2,610,000	1,068,333,333	380,000,000

* Total aerobic bacteria plate count CFU/gram (combined cantaloupe and watermelon)
** Lactic acid bacteria count (combined cantaloupe and watermelon)

[0028] Table 3

Treatment (% CO_2)	Day 3 Off-Flavor		Day 7 *Acceptability	
	CAN	WM	CAN	WM
25-30	**4.3	4.2	***2.4	3.0
55-60	4.0	3.3	2.9	2.7
70-75	4.2	3.8	3.9	3.1

* Acceptability = average of flavor, odor, color and texture observations
** 5 = no off-flavor, 4 = trace, 3 = slight, 2 = moderate, 1 = severe
*** 5 = fresh, 4 = good, 3 = marginal, 2 = unacceptable, 1 = spoiled

Example 2

Experimental Setup

[029] Whole cantaloupe melons were surface sanitized using a steam (Thermal Surface Pasteurization) process. These melons were then hand peeled and cut into ¾ to 1 inch size pieces with sanitized knives. Eight ounces of the cut cantaloupe were weighed into plastic PVC cups laminated with a polybutyl, peelable seal layer (from MAP Systems, Chicago, Ill.). These cups were 4.75" tall, with a 4.2" diameter opening. After filling, the cups were divided into 4 treatments: 1) no initial gas flush but with the same film seal as the other treatments such that a passive modified atmosphere could develop; 2) an initial CO_2-only gas flush (averaging 23.4% CO_2, balance air); 3) an initial, moderately high CO_2 gas flush (averaging 47% CO_2, balance air); and 4) an initial higher CO_2 gas flush (averaging 74.5% CO_2, balance air). The cups were then sealed according to the above treatments with a micro-perforated lidding film supplied by P-Plus, a division of Amcor Inc. The gas flush, sealing packaging machine was a MAP Systems MS-55 (with vacuum). The P-Plus lidding material (52LD50 368 mm) was made of a 2.08 mil polyester to polyethylene laminate base material with an average of 2-3 64-micron perforations per impression/lid as measured during this experiment. According to P-Plus tests, the measured oxygen transmission rate (OTR) of this film would be 167-251 cc of oxygen per package per day. The OTR of the cup material is unknown and believed to be negligible relative to the OTR of the micro-perforated lidding material. All sample cups were then stored at 45-46°F until the evaluations on days 3, 7, 10, 14 and 17.

Results and Conclusions

[030] This example clearly demonstrates the shelf life extending benefits of increasingly high initial CO_2 flushing in combination with a sufficiently gas permeable container for fresh cut cantaloupe at about 46°F. The shelf life observed in this example and others extends well beyond that heretofore reported for fresh cut melons at 46°F (or, for that matter, at 36°F). While some noticeable CO_2 induced off-flavor and off-odor is temporarily detectable, this issue can be managed commercially by applying the appropriate rate of CO_2 dissipation to facilitate the return of normal flavor and odor by the time of the earliest anticipated consumer consumption. This

allows for longer distribution times, broader market serve and better economies of scale for a given fresh cut fruit facility, combined with a consistently more pleasurable eating experience for the consumer.

[031] As shown in Table 4, initial microbial counts were low which enhances the shelf life extending benefits of high CO_2 flushing.

[032] Table 4 — Initial headspace gases and microbial counts

Initial Treatment (% CO_2)	Initial Average CO_2	Initial Average O_2	Initial Average Yeast & Mold Count	Initial Average Total Aerobic Plate Count
None	0.0	20.9	0.0	92.0
25	23.4	15.2	0.0	92.0
50	47.0	10.2	0.0	92.0
75	74.5	4.1	0.0	92.0

[033] Table 5 shows the enhanced reduction in the growth (and/or death) of spoilage organisms after 3 days with increasing initial headspace CO_2. The difference in microbial count between no initial CO_2 flush and 75% CO_2 is a full order of magnitude (1 log reduction).

[034] Table 5 — Headspace gases and microbial counts after 3 days at 46°F

Initial Treatment (% CO_2)	Average CO_2	Average O_2	Average Yeast & Mold Count	Average Total Aerobic Plate Count
None	9.1	13.4	10.0	2122.0
25	17.5	11.9	9.0	658.0
50	20.4	13.0	10.0	400.0
75	25.8	12.7	10.0	230.0

[035] Table 6 reflects slight (temporary) increases in off-odor and off-flavor with increasing initial CO_2 levels; there were no unacceptable scores after 3 days. It is to be noted that if the lidding film had had a slightly higher oxygen transmission rate, the CO_2 level at 3 days would have been slightly lower and there would not have been the slightly elevated odor/flavor scores. This is a good example of how the packaging materials can be manipulated by one skilled in the art to achieve optimum results in the present invention.

[036] Table 6 — Sensory scores after 3 days at 46°F

Initial Treatment (% CO_2)	*Avg. Off Odor	*Avg. Off Flavor	**Avg. Texture (Crispness)	*Avg. Off Color	*** Avg. Acceptability
None	4.5	4.5	4.5	4.5	4.5
25	4.2	4.4	4.5	4.5	4.4
50	4.3	4.4	4.5	4.5	4.4
75	4.0	4.2	4.5	4.5	4.3

* 5 = no off-flavor, off-odor or off-color, 4 = trace, 3 = slight, 2 = moderate, 1 = severe
** 5 = crisp, 4 = firm, 3 = slightly soft, 2 = soft, 1 = mushy
***5 = fresh, 4 = good, 3 = marginal, 2 = unacceptable, 1 = spoiled

[037] The odor/flavor grades are determined by an expert evaluator who smells and tastes blind three samples from each package and assigns a numerical grade on the 1-5 scale. The numbers in the tables are the arithmetic mean of those three scores. The microbiological procedure for quantifying total aerobic bacteria, yeast and mold herein are known in the art and, for example, can be done as follows:

1. Weigh the entire contents of a package (6 oz. to 24 oz. size packages) of fresh cut fruit/produce.
2. Aseptically put the entire package contents (cut fruit) into a sterile stomacher bag with 225 ml. of sterile Butterfields buffer.
3. Seal and place the stomacher bag in the stomacher and stomach/homogenate on “high” for 2 minutes.
4. Serially dilute the sample up to a 10^{-8} dilution by aseptically extracting, using a sterile pipette 1 ml. of homogenate into a test tube containing 9 ml. of sterile Butterfields buffer. Mix thoroughly and continue to dilute from each successively diluted sample to obtain 10^{-8} as the most diluted sample.
1. Place 1 ml. from each of (at least) 5 dilutions (using dilutions estimated (based on experience) to result in plates that grow 25-250 colonies per plate) on (at least) 1 plate each of 3M PETRIFILM™ aerobic plate count (APC) and yeast and mold (Y&M) plates (if counting yeast and molds).
6. Incubate the APC plates for 48 hours at 35°C and the Y&M plates for 3-5 days at 21-25°C.
7. Count and record the number of colonies per plate.

8. Calculate the number of microorganisms per gram of sample using the following formula to determine the average number of colony-forming units (CFU) per gram of original sample:

CFU/g = actual count x 1/dilution x (weight of sample + 225)/weight of sample.

[038] Table 7 shows the enhanced reduction in the growth (and/or death) of aerobic spoilage organisms after 7 days with increasing initial headspace CO_2. The difference in microbial count between no initial CO_2 flush and 75% CO_2 has increased to nearly two orders of magnitude (2 log reduction).

[039] Table 7 — Headspace gases and microbial counts after 7 days at 46°F

Initial Treatment (% CO_2)	Average			
	CO_2	O_2	Yeast & Mold Count	Total Aerobic Plate Count
None	7.8	15.2	13.3	125800.0
25	11.5	14.3	10.0	12160.0
50	14.7	14.3	30.0	12540.0
75	14.9	14.4	15.0	3820.0

[040] Table 8 reflects little difference between treatments in perceived freshness after 7 days at 46°F.

[041] Table 8 — Sensory scores after 7 days at 46°F

Initial Treatment (% CO_2)	*Avg. Off Odor	*Avg. Off Flavor	**Avg. Texture (Crispness)	*Avg. Off Color	*** Avg. Acceptability
None	4.3	4.3	4.0	4.5	4.3
25	4.5	4.5	4.4	4.5	4.5
50	4.1	4.1	4.2	4.5	4.2
75	4.3	4.5	4.5	4.5	4.4

[042] Table 9 shows again the enhanced reduction in the growth (and/or death) of aerobic spoilage organisms and yeast and mold after 7 days, with increasing initial headspace CO_2, after 10 days. It is interesting to note that although the headspace

gases are not very different after the third day, the benefits of the initial CO_2 remain in proportion to the initial levels.

[043] Table 9 — Headspace gases and microbial counts after 10 days at 46°F

Initial Treatment (% CO_2)	Average			
	CO_2	O_2	Yeast & Mold Count	Total Aerobic Plate Count
None	6.7	16.2	10021.8	632000.0
25	11.2	13.6	329.6	137800.0
50	15.3	13.2	9.2	76600.0
75	15.4	13.6	81.4	39000.0

[044] Table 10 reflects a trend of increasing perceived freshness with increasing initial CO_2 levels after 10 days at 46°F. However, the very low initial microbial counts are also providing extended shelf life for all treatments so far.

[045] Table 10 — Sensory scores after 10 days at 46°F

Initial Treatment (% CO_2)	*Avg. Off Odor	*Avg. Off Flavor	**Avg. Texture (Crispness)	*Avg. Off Color	*** Avg. Acceptability
None	4.0	4.1	4.1	4.5	4.2
25	4.4	4.4	4.4	4.5	4.4
50	4.3	4.2	4.2	4.5	4.3
75	4.5	4.5	4.5	4.5	4.5

[046] Table 11 reflects a more obvious trend of increasing perceived freshness with increasing initial CO_2 levels after 14 days at 46°F. The treatment with no initial CO_2 gas flush is judged to have fallen to a marginal degree of freshness.

[047] Table 11 — Sensory scores after 14 days at 46°F

Initial Treatment (% CO_2)	*Avg. Off Odor	*Avg. Off Flavor	**Avg. Texture (Crispness)	*Avg. Off Color	*** Avg. Acceptability
None	3.6	3.5	3.7	4.0	3.7
25	4.0	4.2	4.3	4.5	4.2
50	4.0	4.2	4.3	4.5	4.3
75	4.0	4.5	4.4	4.5	4.4

[048] Table 12 shows how many samples from each treatment had no visible defects after 17 days at 46°F.

[049] Table 12 — Percent of samples visually marketable (out of 20 to 22 remaining) after 17 days at 46°F

Initial Treatment (% CO_2)	Percent Marketable
None	12.0
25	90.0
50	95.0
75	95.0

[050] Table 13 shows average sensory scores for samples that had not been declared unmarketable due to visible defects. As shown in Table 12, only 12% of the samples from the treatment with no initial CO_2 flush were without visible defects (obvious signs of spoilage). The two highest initial CO_2 treatments had the least unmarketable number of samples.

[051] Table 13 — Sensory scores after 17 days at 46°F

Initial Treatment (% CO_2)	*Avg. Off Odor	*Avg. Off Flavor	**Avg. Texture (Crispness)	*Avg. Off Color	*** Avg. Acceptability
None	3.4	3.4	3.5	3.5	3.4
25	4.0	4.3	4.2	4.3	4.2
50	4.1	4.3	4.3	4.4	4.3
75	4.0	4.3	4.3	4.3	4.2

[052] What is claimed is: